CHROM. 3743

Spectrophotometric determination and thin-layer separation of sulfamethazine and procaine penicillin in medicated feeds

Several reports have appeared concerning the separation and determination of sulfamethazine in combination with other sulfa drugs. A colorimetric method of analysis for sulfamethazine in feeds containing procaine penicillin and chlortetracycline recommended by the manufacturer[1] involves a hot alkaline digestion followed by separation using ion-exchange resin and estimation utilizing the BRATTON–MARSHALL[2] reaction. BERG *et al.*[3] used cold alkali for the extraction of the feed and claimed to have recovered about 94.2% of the sulfa drug. It was reported[4] that from a protein-free extract of the medicated feed, both sulfamethazine and procaine penicillin could be directly converted to the colored dye using the BRATTON–MARSHALL reaction and estimated after separating the procaine azo dye. Although this method offers a simple procedure for the determination of sulfamethazine, it cannot, however, be utilized for the estimation of procaine penicillin, since the dye from the antibiotic could not be recovered quantitatively.

Among the methods available for the determination of procaine penicillin, the microbiological assay procedure of MAYERNIK[5] and the colorimetric method using arsenomolybdic acid method[6] are currently in vogue. These are time-consuming and require elaborate laboratory facilities. The present study relates primarily to the thin-layer chromatographic separation of procaine penicillin and sulfamethazine from chlortetracycline and *p*-arsanilic acid when present as a mixture in medicated feeds. Also, it compares the results obtained by this method with those obtained by the procedure recommended by the manufacturer for sulfamethazine. Procaine penicillin and sulfamethazine are estimated spectrophotometrically after the BRATTON–MARSHALL reaction.

In this procedure an aliquot of protein-free ammoniacal dimethylformamide–chloroform–ethanol extract of the feed sample was spotted and developed on an alumina thin-layer chromatoplate using a double elution method[7]. The procaine penicillin and sulfamethazine regions were then quantitatively extracted and the solutions were subjected to the BRATTON–MARSHALL reaction giving a colored dye. The absorbance of the dyes was measured at 545 mμ. By this method, two samples of commercial medicated swine feed were analyzed.

Experimental

One gram of the medicated feed sample was extracted with refluxing ammoniacal dimethylformamide–chloroform–ethanol for 8 h. The warm extract, after filtration through a celite bed, was concentrated under reduced pressure to about 10 ml. The filtrate was diluted with 15 ml absolute ethanol, evaporated to about 5 ml and the operation was repeated once more. The protein-free extract was then quantitatively transferred to a 10-ml volumetric flask and made to volume with the same solvent.

An aliquot (4 × 250 μl) of this extract was spotted on a processed Camag alumina thin-layer chromatoplate (20 cm × 20 cm)[7]. The alumina plate was first developed for 45 min in benzene–chloroform mixture (60:40) followed by drying in air and redeveloping in the same direction for 25 min in a second solvent system, acetonitrile–benzene–

methanol–25% aq. dimethylamine solution (60:30:7.5:2.5). The regions corresponding to procaine penicillin and sulfamethazine were located using shortwave ultraviolet light (R_F values 0.80–0.85 and 0.38–0.42, respectively). The areas containing the drugs were scraped off the plate and extracted with 12 × 10-ml portions of 1% methanolic hydrochloric acid. The extracts were acidified with 2 ml of 6 *N* HCl, diluted with 8 ml of water and evaporated under reduced pressure to about 15 ml. The concentrates were quantitatively transferred to 50 ml volumetric flasks and diazotized with an excess of 1% aq. sodium nitrite solution. The excess nitrite was then decomposed with an excess of 5% aq. ammonium sulfamate solution and the coupling reaction effected by adding an excess of 0.1% aq. solution of N-(1-naphthyl)-ethylenediamine dihydrochloride to give a red dye. The absorbance was measured at 545 mμ using 1-cm quartz cells and a Beckman Model DU spectrophotometer. The concentration was directly read from the standard curve obtained by plotting concentration *vs.* absorbance.

Results and discussion

In attempting to separate cleanly procaine penicillin and sulfamethazine from other medicaments present in finished feeds, several isolated experiments were carried out with a view to selecting a suitable pH for the thin-layer alumina and the solvent system. It was observed that an alumina with a pH 7.2–7.4 afforded adequate separation of these two drugs from one another and also from other drug ingredients. By this procedure the R_F values of authentic samples of procaine penicillin, sulfamethazine, chlortetracycline and *p*-arsanilic acid were 0.75–0.80, 0.35–0.38, 0.00 and 0.00,

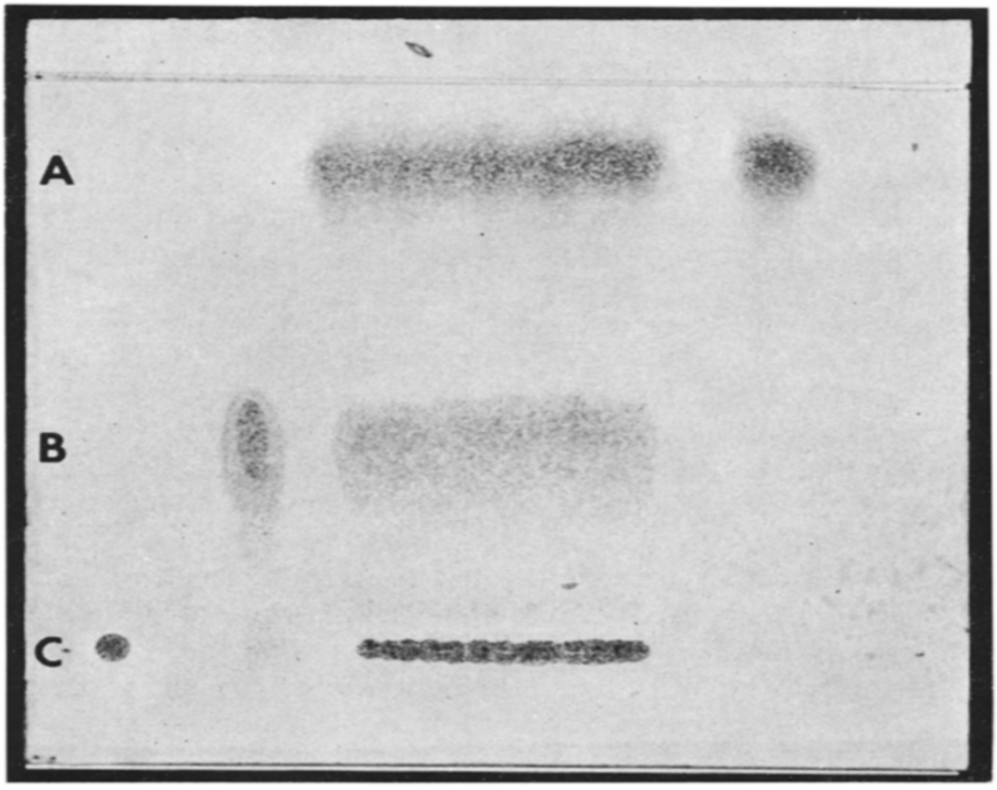

Fig. 1. Chromatogram of a mixture of procaine penicillin (A), sulfamethazine (B), chlortetracycline and *p*-arsanilic acid (C), after the BRATTON–MARSHALL reaction.

TABLE I

ANALYSIS OF FEED SAMPLES FOR PROCAINE PENICILLIN AND SULFAMETHAZINE

Sample	*Sulfamethazine concn. (μg/g)*			*Procaine penicillin concn. (μg/g)*
	Claimed	*This method*	*Manufacturer's method**	*This method*
CF-989	110	107.8	119	132.0
		107.8		118.0
		112.2		113.3
		107.8		126.5
		107.8		135.3
		107.8		126.5
Av.		108.5		125.5
CF-91	110	77.0	77.0	99.0
		71.0		104.5
		82.5		110.0
		79.2		110.0
		73.7		110.0
Av.		76.7		106.7

* We are thankful to Mr. G. D. RITCHIE of this Department for the results.

respectively (Fig. 1). However, the R_F values of the former two drugs when present in feed extracts were slightly altered (0.80–0.85 and 0.38–0.42, respectively). These observed alterations in R_F values were probably due to moisture and other polar compounds extracted from the feed.

Several independent thin-layer chromatographic separations were carried out with known amounts of the drugs which after extraction were estimated as described above. It was observed that the recoveries of procaine penicillin and sulfamethazine were 88–91%.

The results of analysis of the two commercial feed samples after employing an appropriate correction factor (10%) to the observed optical density are presented in Table I.

The values obtained for sulfamethazine in CF-989 indicate that the results are consistent and easily reproducible. By this method, the concentration calculated (108.5 μg/g) is in good agreement with the actual amount (110 μg/g) incorporated in the feed.

On the basis of the above results, it was expected that the values for sulfamethazine estimated by the two methods in CF-91 should be approximately identical. Actually the concentration of sulfamethazine determined by the two methods were 76.7 and 77.0 μg/g of feed. Although the lot of feed represented by sample CF-91 was claimed to contain 110 μg/g of sulfamethazine, it has now been established that the registration information was in error.

In the case of procaine penicillin, the microbiological method which is currently employed, determines the concentration of "active" procaine penicillin at the time of

analysis. This bioassay, however, does not indicate the exact amount of the drug incorporated in the feed because the procaine penicillin is unstable being markedly affected by temperature and the medium. The method described in this communication is developed with a view to determining the amount of the antibiotic which was originally added to the feed.

The samples CF-989 and CF-91 on analysis were found to contain 125.5 and 106.7 μg/g of procaine penicillin, respectively. Since the actual amounts of the drug in these samples were not known, ten standard authentic mixtures containing between 100 and 2000 μg of the drug were estimated by this method. As these results agreed closely with the known concentrations, it may be inferred that the results obtained on Samples CF-989 and CF-91 are substantially correct.

The details of these findings will be reported shortly.

Acknowledgements

We wish to acknowledge our sincere thanks to Drs. K. J. JENKINS and F. B. JOHNSTON of the Research Branch, Canada Department of Agriculture, and Mr. E. J. DOYLE of our Division for their helpful suggestions. Our thanks are due to Miss GAIL STEWART for typing the manuscript.

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Received August 15th, 1968